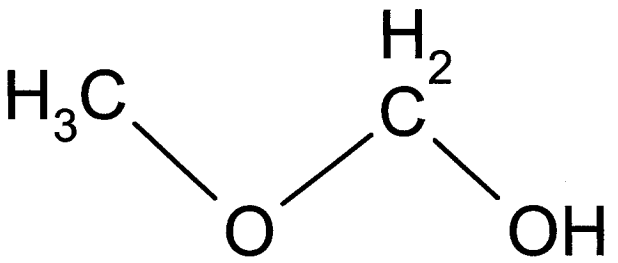
201-15015B

Methoxymethanol

CAS Number 4461-52-3



HPV Data Set

Existing Chemical	:	ID: 4461-52-3
CAS No.	:	4461-52-3
EINECS Name	:	methoxymethanol
EC No.	:	224-722-2
Molecular Formula	:	C2H6O2

Producer related part

Company	:	Celanese Ltd
Technical Contact		Prakash Surana Celanese Ltd. P.O. Box 819063 Dallas, TX 75381 pmsurana@celanese.com (972) 443-4836

Prepared by: Toxicology and Regulatory Affairs, Freeburg IL
CONTACT INFO: Elmer Rauckman (618-539-5280)
rauckman@toxicxolutions.com

Substance related part

Company	:	Celanese Ltd
Creation date	:	20.08.2003
Printing date	:	31.12.2003
Revision date	:	
Date of last update	:	23.12.2003
Number of pages	:	46

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	other: Consulting Toxicologist
Name	:	Toxicology and Regulatory Affairs
Contact person	:	Elmer Rauckman PhD DABT
Date	:	
Street	:	1201 Anise Court
Town	:	Freeburg, IL 62234
Country	:	United States
Phone	:	618-538-5280
Telefax	:	618-539-5394
Telex	:	
Cedex	:	
Email	:	rauckman@toxicsolutions.com
Homepage	:	ToxicSolutions.com

23.12.2003

1.2 SYNONYMS AND TRADENAMES

Formaldehyde methyl hemiacetal

20.08.2003

Hemiformal

20.08.2003

Methanol, hemiformal

20.08.2003

Methanol, methoxy- (8CI9CI)

20.08.2003

Methyl hemiformal

20.08.2003

2.1 MELTING POINT

Remark :
There is no defined melting/freezing point for this mixture.
At temperatures below 65 deg. C, solid polymeric formaldehyde gradually forms.
At temperatures below 0 deg. C, ice crystals can form

In the environment the material will readily dissociate to:
Formaldehyde with a melting point of -92 deg. C (Merck Index, 13th Edition)
Methanol with a melting point of -97.8 deg C (Merck Index, 13th Edition)

Test substance :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint
22.11.2003 (8)

2.2 BOILING POINT

Value : ca. 90 - 95 °C at 1013 hPa

Remark :
The boiling point will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid

In the environment the material will readily dissociate to:
Formaldehyde with a boiling point of -19.5 deg. C @1013 hPa (Merck Index, 13th Edition)
Methanol with a boiling point of 64.7 deg. C @1013 hPa (Merck Index, 13th Edition)

Test substance :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint
22.11.2003 (8)

2.4 VAPOUR PRESSURE

Value : ca. 90 - 95 hPa at 40 °C

Result : The vapor pressure will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid.

In the environment the material will readily dissociate to:

Formaldehyde with a vapor pressure of 5174 hPa @ 25 deg. C (Boublik, T., Fried, V., and Hala, E., The Vapour Pressures of Pure Substances. Second Revised Edition. Amsterdam: Elsevier, 1984. 44 as cited in HSDB)

Methanol with a vapor pressure of 169 hPa @ 25 deg. C (Boublik T et al; The vapor pressures of pure substances: selected values of the temperature dependence of the vapour pressures of some pure substances in the normal and low pressure region. Vol. 17. Amsterdam, Netherlands: Elsevier Sci. Publ 1984. as cited in HSDB)

Test substance :

Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint

22.11.2003 (8)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : ca. -1.4 at 25 °C
pH value :
Method : other (calculated)
Year :
GLP :
Test substance :

Method : Calculated using EPIWIN 3.05 using SMILES input of COCO
Remark :

In the environment the material will readily dissociate to:

Formaldehyde with a log Kow of 0.35 (Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995. 3, as cited in HSDB)

Methanol with a log Kow of -0.77 (ibid.)

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions
EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

22.11.2003 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Remark	:	EPIWIN predicted water solubility of pure material is >1000g/L (EPIWIN 3.05 calculation using SMILES of COCO) In the environment the material will readily dissociate to: Formaldehyde with a water solubility >1000g/L (Merck Index, 13th Edition) Methanol with a a water solubility >1000g/L (Merck Index, 13th Edition)
Result	:	Miscible
Test substance	:	Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.
Reliability	:	(2) valid with restrictions Reliability assigned as 2 since this is experimental data on a water reactive mixture.
Flag	:	Critical study for SIDS endpoint

22.11.2003 (8)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Spectrum of substance	:	lambda (max, >295nm) : nm epsilon (max) : epsilon (295) : 0
DIRECT PHOTOLYSIS		
Halflife t1/2	:	> 1 year
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	cm³/(molecule*sec)
Degradation	:	> 50 % after 15.8 hour(s)
Deg. product	:	
Method	:	other (calculated): APOWIN
Year	:	
GLP	:	
Test substance	:	other TS: Mixture

Method :

As this equilibrium mixture nominally contains methoxymethanol, formaldehyde, methanol and water, and since the initial content of methoxymethanol will be rapidly converted to formaldehyde and methanol, calculations were conducted independently for the three main components.

As there was a discrepancy between the theoretical value of the rate constant for reaction of formaldehyde with hydroxyl radical and an experimental value obtained by Atkinson in 1994, the AOPWIN program was also run on hydrated formaldehyde, which is considered to be in equilibrium with formaldehyde in atmospheres containing water.

Result :

DIRECT PHOTOLYSIS

None of these materials has a chromophore with significant absorption above 295 nm, therefore, direct photolysis is not considered to be an important process in the fate of methoxymethanol preparations.

INDIRECT PHOTOLYSIS

The results of the calculations are shown below. The experimentally derived rate constant for reaction of formaldehyde with hydroxyl radical (Atkinson, 1994) is reconciled by it being a combined rate constant of formaldehyde and hydrated formaldehyde. Formaldehyde is expected to exist in the gas phase as an equilibrium mixture of free and hydrated forms with about a 1:1000 ratio at equimolar concentrations of water. As both the formaldehyde concentration and the atmospheric water concentrations are variables, it is best to assume a range of rate constants and half lives for formaldehyde.

Likewise, methoxymethanol in the vapor phase will react with atmospheric water to produce formaldehyde and methanol. Methanol introduced into the atmosphere, either directly from the mixture or indirectly from hydrolysis of methoxymethanol is considered to exist primarily as the free alcohol in the gas phase when combined with air containing water vapor. The experimentally derived value of the rate constant for the reaction of methanol with hydroxyl radicals is considered more accurate than the predicted value. In addition, as methanol is not as likely to form hydrates, this rate constant is not considered a dependent variable based on atmospheric water content (as is the case with formaldehyde).

Another consideration is polymeric forms of formaldehyde. Due to dilution effects, these are not anticipated to be formed in significant quantity in the vapor phase; however, sublimation of oligomeric formaldehyde from spills of commercial methoxymethanol is possible. The final APOWIN calculation indicates that hydrogen abstraction is very a favorable process for reaction of oligomeric formaldehyde with hydroxyl radical and it will only have an atmospheric half-life on the order of 2 hour. Thus, as it is expected to contribute little to the quantity of material in the air and will not contribute to an extended half-life, it can be ignored relative to atmospheric photodegradation.

In summary, the reaction rate of methoxymethanol or commercial mixtures of formaldehyde, methanol and water with atmospheric hydroxyl radical can be described by the four species listed below.

SPECIES	Half life (12h day 1,500,000 OH molecules/cc)
Methoxymethanol	6.1 hours
Formaldehyde	15.8 hours
Hydroformaldehyde	10.9 hours
Methanol	11.3 hours

As all half-lives are relatively close, the half-life of these mixtures is suffiently well characterized for the purposes of the HPV program as having a range from 6.1 to 15.8 hours

```
Methoxymethanol

AOP Program (v1.90) Results:
============================
SMILES : COCO
CHEM   : Methoxymethanol
MOL FOR: C2 H6 O2
MOL WT : 62.07
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS --
Hydrogen Abstraction      =20.7705 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =20.9105 E-12 cm3/molecule-sec
 HALF-LIFE =     0.512 Days (12-hr day; 1.5E6 OH/cm3)
 HALF-LIFE =     6.138 Hrs


------------------ SUMMARY (AOP v1.90): OZONE REACTION

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)
```

```
Experimental Database:  NO Structure Matches

############################################################


AOP Program (v1.90) Results:
============================
SMILES : O=C
CHEM   : Formaldehyde
MOL FOR: C1 H2 O1
MOL WT : 30.03
------------ SUMMARY (AOP v1.90): HYDROXYL RADICALS-
Hydrogen Abstraction       =8.1300 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =8.1300 E-12 cm3/molecule-sec
   HALF-LIFE =     1.316 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    15.787 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Formaldehyde
  CAS Number:  000050-00-0
 Exper OH rate constant  : 9.37  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
Exper Ozone rate constant: 2.1 E-24  cm3/molecule-sec
Exper NO3 rate constant: 3.2-7.2 E-16  cm3/molecule-sec

_____________________


---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS
SMILES : OCO
CHEM   : HYDRATED FORMALDEHYDE
MOL FOR: C1 H4 O2
MOL WT : 48.04

Hydrogen Abstraction      =11.4415 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =11.7215 E-12 cm3/molecule-sec
   HALF-LIFE =     0.913 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    10.950 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches


####################################################


AOP Program (v1.90) Results:
============================
SMILES : CO
CHEM   : METHANOL
MOL FOR: C1 H4 O1
```

```
MOL WT : 32.04
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -
Hydrogen Abstraction       =0.4760 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =0.6160 E-12 cm3/molecule-sec
   HALF-LIFE =    17.364 Days (12-hr day; 1.5E6 OH/cm3)

----------------- SUMMARY (AOP v1.90): OZONE REACTION --

        ******  NO OZONE REACTION ESTIMATION ******
        (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Methanol
  CAS Number:  000067-56-1
Exper OH rate constant :0.944  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
  Exper Ozone rate constant: --- cm3/molecule-sec
  Exper NO3 rate constant  : --- cm3/molecule-sec

   HALF-LIFE =   11.33 Days (12-hr day; 1.5E6 OH/cm3)

##############################################

SMILES : OCOCOCOCOCOCOCOCO
CHEM   : Polyformaldehyde
MOL FOR: C8 H18 O9
MOL WT : 258.23
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ---
Hydrogen Abstraction      =60.3924 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =60.6724 E-12 cm3/molecule-sec
   HALF-LIFE =     0.176 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     2.115 Hrs

---------------- SUMMARY (AOP v1.90): OZONE REACTION ---

          ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Conclusion :

All half-lives are relatively close, the half-life of these mixtures has a range from 6.1 to 15.8 hours regarding indirect phtolysis in the atmosphere.

Reliability : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

23.11.2003 (4)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : = 6 minute(s) at 25 °C
t1/2 pH7 : = 6 minute(s) at 25 °C

t1/2 pH9 : = .5 minute(s) at 25 °C
t1/2 pH 2 : = 2 minute(s) at 25 °C
Deg. product : yes
Method : other: chemical kinetics
Year :
GLP :
Test substance :

Method :

The rate of decomposition of methoxymethanol was measured by spectroscopically following the trapping of hydrazine derivatives of formaldehyde hydrolysis product. Determinations were made at different pH levels by recording the change in absorbance against time as a function of pH. These data were used to determine the second order rate constants for hydrolysis of methoxymethanol by water, hydrated protons and hydroxyl ion.

Estimates of hydrolysis rates as a function of pH were made by converting the second order rate constants for the hydrolysis into pseudo first-order rate constants at various pH values and estimating the half-life assuming constant water concentration and pH during the hydrolysis and using the usual relationship between first-order rate constants and half-life.

Result :

The second order rate constants derived for the hydrolysis are:

Reaction with water: k(w) = 3.27 E-5 M^-1 sec^-1
Reaction with H+: k(H) = 0.58
Reaction with OH-: k(OH) = 2.34 E3

Converting these to pseudo-first order rate constants and extrapolation half-lives the following t1/2 are obtained:

	half-life					
Rxn with	2	4	6	7	8	9
Water	6 min	6 min	6 min	6 min	6 min	6 min
Acid	2 min	3.3 hr	333hr	>1000hr	>1000 hr	>1000
Base	>1hr	>1hr	490min	49 min	4.9 min	30 sec

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Conclusion :

Methoxymethanol has a maximum half-life in water of 6 minutes at 25°C. Its pH dependency displays a broad peak from about pH 3 to pH 8. Above or below this range of pH the reaction with acid or base predominates over an already facile reaction with water producing and even shorter half-life. Reaction with base is faster than reaction with acids.

Reliability : (1) valid without restriction

Calculated from peer-reviewed experimental chemical reaction rate constants.

Flag : Critical study for SIDS endpoint
23.12.2003 (7)

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C

Result :

This preparation as typically sold, transported and used is an equilibrium mixture of formaldehyde:methanol:water in a mole ratio of about 3.3:2.0:1.0. The chemical makeup of this mixture is such that there is formally an excess of formaldehyde; however it exists primarily as a series of methanol hemiacetals and hydrates. When added to water, the equilibrium shifts rapidly toward formaldehyde hydrates and methanol.

Methanol is a simple alcohol and alcohols are one of the chemical groups considered stable to hydrolysis (Harris, 1990).

Formaldehyde is known to be water reactive reversibly forming a hydrate (HO-CH2-OH) the equilibrium constant for formaldehyde hydrate formation is > 1000 (Vollhardt, 1987). Thus, formaldehyde is known to be stable indefinitely in water, existing 99.9% as a hydrated species.

Harris, J.C. in Lyman W., Reehl, W. and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6

Vollhardt, Peter (1987) Organic Chemistry WH Freeman publisher NY p 637

Test substance :

Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Estimated values based on sound chemical principles are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

23.12.2003 (11) (17)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year :

Method :

Since this mixture contains methoxymethanol, formaldehyde and methanol, and since the initial concentration of methoxymethanol will be readily converted to formaldehyde and methanol the calculations had to be conducted independently.

The actual physical properties for formaldehyde and methanol were input while they were allowed to be calculated for pure methoxymethanol (as they are not known with accuracy). EPIWIN was allowed to set the values for half-lives in various media. Emissions were set to equal values for air

water and soil (the EPIWIN default) for consistency.

SMILES inputs
COCO
CO
C=O

Result :

The calculations indicate that all three major components distribute primarily to water followed closely by soil. Only methanol indicates that it we distribute to air more than a few percent. As this is a variable mixture in actual production and use, and as these materials have high water solubility and biodegradability these estimates are adequate to understand the approximate distribution of the material in the environment.

```
Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : Methoxymethanol
  Molecular Wt: 62.07
  Henry's LC  : 1.47e-006 atm-m3/mole (Henrywin program)
  Vapor Press : 32 mm Hg  (Mpbpwin program)
  Log Kow     : -1.4  (Kowwin program)
  Soil Koc    : 0.0163  (calc by model)

         Concentrat Half-Life Emissions
           (percen)   (hr)     (kg/hr)
   Air       1.92      12.3      1000
   Water     54.8       360      1000
   Soil      43.2       360      1000
   Sediment  0.0913    1440        0

            Fugacity  React  Advect Reaction  Advection
             (atm)    kg/h) (kg/h)  (percent) (percent)
   Air       6.12e-011  878    155     29.3      5.18
   Water     5.24e-011  852    442     28.4      14.7
   Soil      1.53e-009  672     0      22.4      0
   Sediment  4.36e-011  .355   .0147   .0118     .000492

   Persistence Time: 269 hr
   Reaction Time:    336 hr
   Advection Time:   1.35e+003 hr
   Percent Reacted:  80.1
   Percent Advected: 19.9

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      12.28
      Water:    360
      Soil:     360
      Sediment: 1440
        Biowin estimate: 3.213  (weeks)

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004



METHANOL

Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : methanol
  Molecular Wt: 32.04
  Henry's LC  : 4.55e-006 atm-m3/mole (Henry database)
  Vapor Press : 127 mm Hg  (user-entered)
  Log Kow     : -0.77  (Kowwin program)
  Soil Koc    : 0.0696  (calc by model)

         Concentration   Half-Life    Emissions
           (percent)        (hr)       (kg/hr)
   Air       13             272          1000
   Water     47.2           208          1000
   Soil      39.7           208          1000
   Sediment  0.0705         832          0
```

```
                Fugacity  Reaction  Advection  Reaction  Advection
                 (atm)    (kg/hr)   (kg/hr)   (percent)  (percent)
   Air       5.96e-010  199       782        6.64      26.1
   Water     2.01e-010  943       283        31.4      9.44
   Soil      6.22e-009  792       0          26.4      0
   Sediment  1.5e-010   .352      .00846     0.0117    0.000282

   Persistence Time: 200 hr
   Reaction Time:    310 hr
   Advection Time:   563 hr
   Percent Reacted:  64.5
   Percent Advected: 35.5

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      271.9
      Water:    208.1
      Soil:     208.1
      Sediment: 832.3
        Biowin estimate: 3.288  (days-weeks  )

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

```
FORMALDEHYDE

Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : Formaldehyde
  Molecular Wt: 30.03
  Henry's LC  : 3.37e-007 atm-m3/mole (Henry database)
  Vapor Press : 3.89e+003 mm Hg  (user-entered)
  Liquid VP   : 2.04e+004 mm Hg  (super-cooled)
  Melting Pt  : 97.8 deg C (user-entered)
  Log Kow     : 0.35  (user-entered)
  Soil Koc    : 0.918  (calc by model)

          Concentration   Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       2.7             27.4         1000
   Water     51.3            360          1000
   Soil      45.9            360          1000
   Sediment  0.0871          1.44e+003    0

             Fugacity Reaction Advection Reaction  Advection
              (atm)   (kg/hr)   (kg/hr)  (percent) (percent)
   Air       1.98e-010  614      243       20.5      8.09
   Water     2.59e-011  887      461       29.6      15.4
   Soil      7.99e-010  795      0         26.5      0
   Sediment  2.15e-011  0.377    0.0157    0.0126    0.000522

   Persistence Time: 300 hr
   Reaction Time:    391 hr
   Advection Time:   1.28e+003 hr
   Percent Reacted:  76.5
   Percent Advected: 23.5

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      27.41
      Water:    360
      Soil:     360
      Sediment: 1440
        Biowin estimate: 3.155  (weeks)

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

22.11.2003 (6)

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other: not pre-acclimated inoculum
Contact time	:	
Degradation	:	= 90 (±) % after 28 day(s)
Result	:	readily biodegradable
Deg. product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	1990
GLP	:	no
Test substance	:	other TS
Remark	:	Result adopted from SIDS 2003 document. Material was agreed to be readily biodegradable at the SIAM meeting
Test substance	:	Formaldehyde CASNO 50-00-0
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

22.11.2003 (10)

Type	:	aerobic
Inoculum	:	activated sludge, domestic, non-adapted
Contact time	:	
Degradation	:	= 50 - 80 (±) % after 6 day(s)
Result	:	
Remark	:	This robust summary was adopted from the Methanol HPV document. Please see the Methanol HPV document for additional studies. Methanol has been well studied in biodegradation assays of several types and the weight of evidence indicates it is readily biodegradable.
Test substance	:	Methanol
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

22.11.2003 (15)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	other: ECOSAR Estimate
Species	:	other: freshwater fish
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	ca. 45 calculated
Method	:	other: Estimate
Year	:	
GLP	:	
Test substance	:	

Method :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the LC50 for fish. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the LC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted LC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into fish tissues due to its Kow.

Result :

The ECOSAR estimate (in its entirety) is presented for completeness but the LC50 for methoxymethanol is estimated at 55% (the weight percent of formaldehyde in the mixture) of the published LC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism    Duration End Pt   mg/L
==================== =========== ======== ====   ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
Neutral Organics   : Green Algae   96-hr   EC50  31468.400
Neutral Organics   : Fish          30-day  ChV    5381.146
Neutral Organics   : Daphnid       16-day  EC50    709.373
Neutral Organics   : Green Algae   96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)    96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp  96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm     14-day  LC50   4256.716
```

Estimate based on formaldehyde toxicity 1/55% of 24.8 = 45 mg/L for freshwater fish.

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Type : flow through
Species : Ictalurus melas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 24.8 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: acute toxicity test; "flow through bioassay"
Year : 1977
GLP : no
Test substance : other TS: formalin, commercial grade, 37%

Method :

fingerling; pH 6.5, water hardness 8, water temperature 12 degrees Centigrade

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

Result :

Test result: 62.1 µl/l formalin (solution 37%)

Test substance :

Formaldehyde CASNO 50-00-0

Reliability : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

23.11.2003 (1)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	other: estimate
Species	:	other: freshwater invertebrate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	ca. 10.5 calculated
Method	:	other: Estimate
Year	:	
GLP	:	
Test substance	:	

Method :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for daphnia. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to invertebrates (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to daphnids, formaldehyde is the species that will determine the acute toxicity of this mixture to invertebrates. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the EC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted EC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into invertebrates tissues due to its Kow.

Result :

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the published EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
```

```
-----------------------------
Neutral Organics
                                                    Predicted
ECOSAR Class          Organism   Duration End Pt   mg/L
==================== =========== ======= ====   ==========
Neutral Organic SAR: Fish         14-day  LC50   76256.125
(Baseline Toxicity)

Neutral Organics   : Fish         96-hr   LC50   72256.133
Neutral Organics   : Fish         14-day  LC50   76256.125
Neutral Organics   : Daphnid      48-hr   LC50   61217.387
Neutral Organics   : Green Algae  96-hr   EC50   31468.400
Neutral Organics   : Fish         30-day  ChV     5381.146
Neutral Organics   : Daphnid      16-day  EC50     709.373
Neutral Organics   : Green Algae  96-hr   ChV      441.037
Neutral Organics   : Fish  (SW)   96-hr   LC50    3197.973
Neutral Organics   : Mysid Shrimp 96-hr   LC50    2.36e+005
Neutral Organics   : Earthworm    14-day  LC50    4256.716

Estimate based on formaldehyde toxicity 1/55% of 5.8  = 10.5 mg/L for
daphnids.
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Type : other: According to OECD standard
Species : Daphnia pulex (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 5.8 measured/nominal
EC10 : = measured/nominal
EC90 : = 16.8 measured/nominal
Limit Test : no
Analytical monitoring : no data
Method :
Year :
GLP : no data
Test substance : other TS: Formaldehyde

Result :

EC50 (48 h) = 4.3 - 7.8 (confidence limit)

Test condition :

test temperature 20 +/- 1 °C,
the standard stock solutions were prepared according to Standard Methods: APHA-AWWA-WEF, 1992 and Leithe, 1974, daphnids cultured in 3-L-aquariumsand beakers were illuminated 12 hr per day

Test substance :

Formaldehyde 37 % v/v

Reliability : (2) valid with restrictions

accepatable study, meets basic scientific principles

23.11.2003 (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae: green generic
Endpoint	:	biomass
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC10	:	= calculated
Method	:	other: Estimation
Year	:	2003
GLP	:	
Test substance	:	

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to algae (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to algae, formaldehyde is the species that will determine the acute toxicity of this mixture to aquatic plants. In recognition of this, two robust summaries flagged as "critical for SIDS endpoint" have been adopted from the formaldehyde SIDS document for use in the estimation of methoxymethanol toxicity to aquatic plants. The reader is referred to the formaldehyde SIDS document for additional supporting studies.

The critical studies were a long duration (192 hour) and a short duration (24 hour) study using the same species. Different endpoints were used and the estimated toxicity of methoxymethanol was calculated by taking the geometric mean of the toxic threshold value from the 192-hour study and the EC50 of the 24-hour study and setting this as the 72-hour EC50 for formaldehyde. Although there is no known scientific precedent for this calculation, it recognizes that the true value of the 72-hour EC50 for formaldehyde is lower than the 24-hour EC50. It is also recognized that formaldehyde undoubtedly reacted with the algae reducing its concentration greatly in the 192-hour study and probably in the 24-hour study. These data are considered acceptable for the estimate as ODED has recently accepted this data set for formaldehyde and as it would be impossible to accurately determine the EC50 of formaldehyde due to its reactivity and volatility.

Result :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for algae. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the calculated EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism    Duration End Pt   mg/L
==================== =========== ======= ====   ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
Neutral Organics   : Green Algae   96-hr   EC50  31468.400
Neutral Organics   : Fish          30-day  ChV    5381.146
Neutral Organics   : Daphnid       16-day  EC50    709.373
Neutral Organics   : Green Algae   96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)    96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp  96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm     14-day  LC50   4256.716

Estimate based on formaldehyde toxicity 1/55% of 6.0 (geometric mean
of 196-hour EC03 and 24-hour EC50)   = 11.5 mg/L for green algae.
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Species : Scenedesmus quadricauda (Algae)
Endpoint : biomass
Exposure period : 192 hour(s)
Unit : mg/l
EC03 : = .88 measured/nominal
Method : other: Static Cell Multiplication Inhibition Test
Year : 1978
GLP : no
Test substance : other TS: Formaldehyde

Result :

Toxicity Threshold : 2.5 mg/l 35% formalin
0.88 mg/l Formaldehyde

Toxic threshold is defined in this investigation as the concentration of test substance causing 3 % inhibition of cell multiplication compared to untreated controls.

Test condition :

Test vessel: Kapsenberg cultivation tubes (18 x 180mm)

Concentration of stock solution: not indicated

Pre-treatment of test solution: neutralisation if necessary

Inoculum:cell density adjusted to TE/F = 20 formazin turbidity equivalents at 578nm)

Test volume: 10 ml

Dilution:1:2
Number of test replicates:3

Number of control replicates:1

Illumination:constant artifical light (Osram L 40/30)

Temperature: 27 °C

Agitation: once daily

Measurements:photometric determination of cell density at578 nm after 192 h of exposure

Test substance :

Formalin (35% solution)

Reliability : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

23.11.2003 (2)

Species : Scenedesmus quadricauda (Algae)
Endpoint : other: Oxygen uptake and produstion
Exposure period : 24 hour(s)
Unit : mg/l
EC10 : = 3.6 measured/nominal
EC50 : = 14.7 measured/nominal
EC90 : = 60.3 measured/nominal
Method :
Year :
GLP : no data
Test substance : other TS: Formaldehyde

Method :

Toxicity to algae was evaluated by measuring the oxygen production and consumption rates following exposure to the test media and calculating the 24-hr net assimilation by the algae.

The oxygen production and consumption rates were measured on Warburg apparatus (type 85G, B.Braun, Germany)

The effective concentrations were calculated using linear regression analysis.

Remark :

Short duration

Test condition :

test temperature 20 +/- 1 °C,
Standard stock solutions were prepared according to Standard Methods: APHA-AWWA-WEF, 1992 and Leithe, 1974,cultured in the nutrient solution prepared according to Holm Hansen (Bringmann and Kühn, 1980) under continuous illumination (3000 lx)

Test substance :

Formaldehyde (37% solution in water)

Reliability : (2) valid with restrictions

accepatable study, meets basic scientific principles

23.11.2003 (16)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 1269 mg/kg bw
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male/female
Number of animals	:	
Vehicle	:	water
Doses	:	707, 1000, 1414, 2000, 2828
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

Specific guideline not specified.

The test substance in distilled water (dissolved just before administration) was administered by gavage to groups of five rats of each sex that had been fasted overnight. Doses, based on a range-finding study, were 707, 1000, 1414, 2000, and 2828 mg/kg. The volume of administration was 10 ml/kg and feed was not given for approximately three hours after administration. Purity was not determined when the test solution was prepared.

General conditions of animals were observed on the day of administration at 5 minutes, 15 minutes, 30 minutes, 1 hour, 3 and 6 hours after dosing, and once a day for a period of 14 days. Body weight was measured just before treatment, and on days 3, 7 and 14. Dead animals were necropsied promptly after discovery. After the 14-day observation period, surviving animals were sacrificed and examined. The LD50 was computed using the probit method.

Result :

Mortality was observed as indicated in the table below:

Dose	MORTALITY	
	Males	Females
0	0/5	0/5
707	0/5	0/5
1000	1/5	1/5
1414	3/5	2/5
2000	5/5	4/5
2828	5/5	5/5

Most deaths were within an hour of administration

LD50 values were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

Clinical Observations: Reduced spontaneous activity, slow breathing and blepharoptosis were observed across all groups; groups at 2000 mg/kg and

above showed lying down, gasping and clonic seizures. Salivation was reported among all groups other than the female 707 and 1414 mg/kg groups. Other symptoms included lacrimation, red lacrimation, red nasal drainage and raising of the tail.

Body Weights: Some of the surviving animals in 1414 mg/kg and 2000 mg/kg groups showed weight loss on the third day post-administration, but gained weight thereafter. Surviving animals of the other groups gained weight throughout the period of observation.

Necropsy: Animals dying from treatment showed atrial enlargement, pulmonary congestion/edema, and congestion/ edema/hemorrhaging/erosion of glandular stomach mucous membrane. Among surviving animals, adhesions of stomach and liver, thickening of the anterior stomach mucous membrane and erosion/ulceration of glandular stomach mucus membrane were noted in the groups at dose levels of 2000 and 2828 mg/kg.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Conclusion :

The LD50 values were:

Males: 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg)
Females: 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

No specific target organs were identified.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (14)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	41 to 47 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	12, 60 or 300 mg/kg-day
Control group	:	yes, concurrent vehicle

Method : other: OECD Guideline 422
Year :
GLP : yes
Test substance :

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant

difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document :

Organ Wts.bmp
Hematol-ps.bmp
Biochem-ps2.bmp
Histopath.bmp

Table 3 Absolute and relative organ weight of rats treated orally with methoxymethanol in combined repeat dose and reproductive/ developmental toxicity screening test

Sex	Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
Male					
	No of animals	10	10	10	9
	Body weight (g)	445 ± 26.1	440 ± 25.8	449 ± 32.5	393 ± 45.0*
	Absolute organ weight				
	Thymus (mg)	358 ± 60.0	415 ± 80.7	335 ± 44.3	287 ± 90.9
	Liver (g)	12.27 ± 1.547	11.89 ± 1.397	12.21 ± 1.373	11.45 ± 1.195
	Kidneys (g)	2.86 ± 0.250	2.93 ± 0.305	2.82 ± 0.236	2.72 ± 0.269
	Testes (g)	3.39 ± 0.227	3.15 ± 0.147	3.21 ± 0.344	3.27 ± 0.277
	Epididymides (g)	1.26 ± 0.121	1.22 ± 0.087	1.25 ± 0.127	1.23 ± 0.120
	Relative organ weight				
	Thymus (mg%)	80 ± 10.7	95 ± 19.0	75 ± 9.0	72 ± 19.3
	Liver (g%)	2.75 ± 0.213	2.70 ± 0.209	2.71 ± 0.133	2.92 ± 0.233
	Kidneys (g%)	0.64 ± 0.028	0.67 ± 0.057	0.63 ± 0.041	0.69 ± 0.046
	Testes (g%)	0.75 ± 0.081	0.72 ± 0.056	0.72 ± 0.049	0.84 ± 0.112
	Epididymides (g%)	0.28 ± 0.025	0.28 ± 0.024	0.28 ± 0.018	0.32 ± 0.026
Female					
	No of animals	10	10	10	9
	Body weight (g)	313 ± 14.8	315 ± 22.4	312 ± 19.7	310 ± 14.8
	Absolute organ weight				
	Thymus (mg)	199 ± 69.2	216 ± 71.0	236 ± 101.8	185 ± 31.9
	Liver (g)	14.25 ± 0.945	13.84 ± 1.876	13.83 ± 0.567	15.10 ± 1.477
	Kidneys (g)	2.10 ± 0.255	2.11 ± 0.249	2.29 ± 0.574	2.01 ± 0.130
	Relative organ weight				
	Thymus (mg%)	63 ± 21.2	69 ± 24.2	75 ± 29.0	60 ± 9.6
	Liver (g%)	4.55 ± 0.255	4.38 ± 0.370	4.45 ± 0.320	4.87 ± 0.446
	Kidneys (g%)	0.67 ± 0.037	0.67 ± 0.067	0.71 ± 0.176	0.65 ± 0.041

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05.

Table 1 Hematology of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test.

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of aminals	10	10	10	9
RBC ($\times 10^4$/mm^3)	813 ± 6.0	815 ± 41.7	820 ± 24.7	755 ± 52.0*
Hematocrit (%)	43.7 ± 1.00	43.6 ± 1.16	43.6 ± 1.18	38.7 ± 4.66**
Hemoglobin (g/dl)	15.5 ± 0.41	15.4 ± 0.53	15.6 ± 0.32	13.5 ± 1.92**
Reticulocyte (‰)	25 ± 3.5	26 ± 4.4	26 ± 2.8	45 ± 18.7**
MCV (μm^3)	53.8 ± 1.33	53.6 ± 1.88	53.1 ± 1.62	51.2 ± 3.92
MCH (pg)	19.1 ± 0.60	19.0 ± 0.71	19.0 ± 0.38	17.8 ± 1.81
MCHC (%)	35.5 ± 0.43	35.4 ± 0.48	35.7 ± 0.53	34.7 ± 1.07
Platelet ($\times 10^4$/mm^3)	102.8 ± 11.02	103.3 ± 13.55	106.6 ± 17.65	127.4 ± 30.09**
WBC ($\times 10^2$/mm^3)	104 ± 31.4	107 ± 29.8	104 ± 20.8	103 ± 33.4
Differential leukocyte counts (%)				
Lymphocytes	78 ± 8.6	81 ± 6.2	83 ± 6.0	76 ± 8.5
Neutrophils				
segmented	16 ± 7.8	12 ± 5.2	11 ± 6.0	19 ± 6.2
band	0 ± 0.3	1 ± 0.9	1 ± 0.8	1 ± 0.5
Eosinophils	1 ± 0.5	1 ± 0.9	1 ± 1.2	1 ± 0.7
Basophils	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Monocytes	5 ± 1.9	5 ± 1.6	4 ± 2.0	4 ± 4.1

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 2 Blood chemistry of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of aminals	10	10	10	9
GOT (IU/l)	83 ± 14.1	84 ± 13.1	78 ± 11.6	93 ± 11.8
GPT (IU/l)	27 ± 5.6	26 ± 3.5	26 ± 4.0	33 ± 9.7
γ-GTP (IU/l)	0 ± 0.0	0.1 ± 0.316	0 ± 0.0	0 ± 0.0
ALP (IU/l)	283 ± 32.6	245 ± 54.3	233 ± 50.9	199 ± 53.8
Total bilirubin (mg/dl)	0.11 ± 0.032	0.05 ± 0.053**	0.10 ± 0.00	0.09 ± 0.033
Urea nitrogen (mg/dl)	18.5 ± 2.08	18.8 ± 2.64	18.7 ± 2.58	17.1 ± 4.06
Creatinie (mg/dl)	0.5 ± 0.03	0.5 ± 0.03	0.5 ± 0.06	0.4 ± 0.05
Glucose (mg/dl)	126 ± 8.1	128 ± 13.3	132 ± 13.7	115 ± 23.8
Total chol. (mg/dl)	75 ± 21.8	65 ± 14.8	69 ± 11.0	69 ± 8.9
Triglyceride (g/dl)	58 ± 28.4	49 ± 20.8	74 ± 36.1	64 ± 25.0
Total protein (g/dl)	6.69 ± 0.187	6.33 ± 0.476	6.46 ± 0.260	5.61 ± 0.312**
Albumin (g/dl)	3.71 ± 0.083	3.61 ± 0.229	3.70 ± 0.110	3.38 ± 0.157**
A/G ratio	1.25 ± 0.050	1.33 ± 0.074	1.34 ± 0.058	1.53 ± 0.190**
Ca (mg/dl)	9.4 ± 0.22	9.3 ± 0.32	9.3 ± 0.21	8.9 ± 0.17**
Inorganic phos. (mg/dl)	7.4 ± 0.46	7.6 ± 0.37	7.5 ± 0.45	7.5 ± 0.66
Na (meq/l)	144 ± 0.6	144 ± 1.0	144 ± 0.9	144 ± 0.8
K (meq/l)	4.5 ± 0.17	4.5 ± 0.25	4.5 ± 0.10	4.6 ± 0.52
Cl (meq/l)	105 ± 1.3	106 ± 2.0	105 ± 1.0	107 ± 1.3**

Values are expressed as Mean ± S.D.
Significantly different from control group; **: P<0.01.

Table 5 Summary of histopathological findings in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Organ	Sex:	Male				Female			
	Dose level (mg/kg):	0	12	60	300	0	12	60	300
findings	No. of animals:	10	10	10	9	10	10	10	10
Stomach									
Ulcer		0	0	0	5	0	0	0	8
Erosion		0	0	2	3	0	0	0	2
Focal regenerative change of gastric gland		0	0	3	6	0	0	0	5
Inflammatory cell infiltration in submucosal layer		0	0	0	9	0	0	0	5
Duodenum									
Thickening of mucosa		0	0	0	6	0	$	$	0
Adrenals									
Hypertrophy of zona fasciculata and zona reticularis		0	0	0	2	0	0	0	0
Kidenys									
Basophilic change of the tubular epithelium		0	$	$	0	2	$	1/1 #)	0
Liver									
Peripheral fatty change		0	$	1/1	0	0	$	$	0
Focal necrosis		0	$	$	1	0	$	$	0
Skin									
Erosion		$	1/1	$	$	$	$	$	$

$: Not examined, #:Number of animals with lesion / Number of animals examined.

Conclusion :

Toxic effects related to administration of the test substance were observed primarily in the digestive tract and are considered to result primarily from the irritating property of the test substance. For males effects were seen at 60 mg/kg-day and above. For females, effects were seen only at the high dose.

Regarding hematology, changes in RBC's (reduced number), reticulocytes and platelets (increased) were only seen in the high-dose males. These effects may have been related to gastric ulceration and subsequent loss of blood.

Regarding clinical chemistry, effects were found only for the high-dose males. The reduction in total protein and albumin and the albumin/globulin ratio are also consistent with gastric ulceration and subsequent loss of blood.

Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood.

The following effect levels are assigned:
LOAEL
60 mg/kg-day (males)
300 mg/kg-day (females)

NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint
01.12.2003 (9)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium TA100, TA1535, TA98, TA 1537 and E coli WP2 uvrA
Test concentration	:	Up to 625 micrograms/plate for Salmonella and 2500 micrograms/plate for E coli.
Cycotoxic concentr.	:	Salmonella 500 micrograms/plate and above E coli 2500 micrograms/plate and above
Metabolic activation	:	with and without
Result	:	positive
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

Using the plate incorporation method, the following bacterial strains were exposed to test material in the presence and absence of S9 mix (prepared from Sprague-Dawley type male rats induced by concurrent administration of phenobarbital and 5, 6-benzoflavone):

Salmonella typhimurium TA100
Salmonella typhimurium TA1535
Escherichia coli WP2 uvrA
Salmonella typhimurium TA98
Salmonella typhimurium TA 1537

The study was a triple plate, independent repeat design. A preliminary toxicity study was conducted using five concentrations of test material from 50 to 5000 microgram per plate. The test material was determined to be cytotoxic to Salmonella at 500 micrograms per plate and above and cytotoxic to E coli at 1500 micrograms per plate and above.

Evaluation criteria were as follows: When the number of revertant colonies on the plate containing the test substance was found to be more than two times that of the negative control, and at the same time, when reproducibility or dose dependency for its increase is seen in more than one strain of bacteria by either the direct or metabolic activation method, the said test substance was determined to be mutagenic (positive) for those strains.

Result :

Only strains TA100 and TA98 showed increases in revertants and data are shown in this robust summary only for these two strains

The tables below show the mean of the revertants from three replicate plates

TA100	Trial 1		Trial 2	
Dose	-S9	+S9	-S9	+S9
0	129	134	123	121
19.53	96	140	141	130
39.06	98	154	193	183
78.12	116	165	338	369
156.2	199	238	228	264
312.5*	175	129	16	96
625*	2	19	0	0

TA98	Trial 1		Trial 2	
Dose	-S9	+S9	-S9	+S9
0	17	27	19	23
19.53	21	31	29	38
39.06	28	34	55	39
78.12	59	44	74	47
156.2	95	58	53	48
312.5*	38	33	0	10
625*	0	0	0	0

* = Bacterial growth inihibition

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Conclusion :

TA100 and TA98 showed numbers of revertants and dose dependency consistent with the evaluation criteria for a positive result. The test material is considered positive for mutagenic activity in this system under these conditions.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (13)

Type : Chromosomal aberration test
System of testing : Chinese hamster lung cells
Test concentration : 0.005 to 0.032 mg/ml
Cycotoxic concentr. : 0.02 or 0.032 in the presence of S9 mix
Metabolic activation : with and without
Result : positive
Method :
Year :
GLP :
Test substance :

Method :

Frozen Chinese hamster lung (CHL) cells derived from Chinese hamsters (obtained February, 1988, in the fourth successive generation from Research Resource Bank (JCRB)) were thawed and used for the test within the tenth successive generation. Eagle MEM culture medium with 10% fetal calf serum was used as the growth media.

CHL cells (20,000) were seeded into 5 ml culture medium in a flask (Croning 25 cm2) and was incubated in a CO2 incubator (5% CO2) at 37?.

For the direct method, the test substance was added on the 3rd post-seeded

day and the samples were exposed to the test substance for either 24 or 48 hours. For metabolic activation with and without the presence of S9 mix, the samples were exposed for 6 hours on the 3rd post-seeded day and upon completion of the exposure they were further cultured in fresh media for an additional 18 hours.

Dilutions of test substance were freshly prepared in acetone before each use. Containers with caps were used to minimize any changes occurring from volatilization of the substance during the preparation and handling. The test substance was dissolved in the solvent and then further diluted acetone serially to obtain the desired concentrations of the test solution. The test solution was then added to the culture media at 0.5% (v/v) for all testing. Analytical measurements of the test substance in acetone dilutions were conducted and all concentration except the 1.00 mg/ml concentration were within the acceptable range (85% of the added amount). The deviation form target concentration in the 1.0 mg/ml dilution was attributed to volatility of the test material.

Cytotoxicity was determined by adding different concentrations of MM to the cultures using the direct, the indirect and the indirect with S-9 culture conditions. Growth inhibition was measured by determining the mitotic index. The concentration exerting 50% growth inhibition (50% reduction of mitotic index) was found to be 0.020 mg/ml for the direct method while the 50% inhibitory concentrations for metabolic activation with and without S-9 mix were 0.032 mg/ml and 0.019 mg/ml, respectively. The source of the S9 was not reported.

Dose selection: Based on the results from the cell growth inhibition test, the high concentrations of the test substance were determined to be 0.020 mg/ml for the direct method and 0.032 mg/ml and 0.020 mg/ml for the metabolic activation method with S9 mix and without S9 mix, respectively. Half strength of each corresponding high concentration was used as the medium concentration and 1/4 as the low concentration.

Two hours prior to the completion of incubation, Colcemid was added to the culture media so that its final concentration was approximately 0.1µg/ml. Six slides were prepared from each petri dish and were stained with 3% Giemza solution for 10 minutes.

Slides were coded and read blind. The chromosomal analysis was based on the classification by the Japan Environmental Mutagen Association, Mammalian Mutation Study (MMS) Subcommittee, and structural aberrations of chromosome or chromatid such as gaps, breaks and exchanges, as well as polyploid cells were scored. For structural aberrations, 200 cells per group and for poliploid cells, 800 metaphase cells per each group were analyzed.

Statistics analysis was conducted using Fisher's exact test to determine the significance of differences in the number of cells with chromosomal aberrations between the solvent control groups and the groups treated with the test substance, and the positive control groups. The potential of the test substance to induce chromosomal aberrations was determined based on the criteria established by Ishidate et al. where the percentage of cells with chromosomal aberrations less than 5% is considered negative, while a percentage of more than 5% and below 10% is considered equivocal and if greater than 10% it is considered positive.

Result :

Results of chromosomal analysis using the direct method are shown in Table 1. As the result of exposure to methoxymethanol for 24 hours, the percentage cells with chromosomal structural aberrations and polyploid cells increased significantly in a concentration dependent relation. Methoxymethanol was determined to be positive for structural aberrations. The evaluation of polyploid cells was equivocal. With the 48-hour exposure, chromosomal structural aberrations were induced in 6% of the cells (including gaps) in the high concentration group (0.020mg/ml) indicating an equivocal result. There were also significant increases in the number of polyploid cells in the low concentration group (0.005mg/ml) and in the high concentration group (0.020 mg/ml) indicating an equivocal result for the high concentration group.

Results of chromosomal analysis using metabolic activation are shown in Table 2. Following the application of methoxymethanol, the high concentration groups with 6-hour exposure with and without the presence of S9 mix revealed chromosomal aberrations (including gaps) in 16.5%- 26% of the studied cells indicating a positive result. Further, there was a significant increase in the appearance frequency of polyploid cells among the medium and high concentration groups indicating an equivocal result.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : CA Tab-1.bmp
CA Tab-2.bmp

Table 1 Chromosome analysis of Chinese hamster cells (CHL) continuously treated with methoxymethanol ** without S9 mix

Group	Concent-ration (mg/ml)	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations: gap	ctb	cte	csb	cse	f	mul[2]	total	Others[3]	No. of cells with aberrations: TAG	(%)	TA	(%)	Polyploid[4] (%)	Judgement[5]: SA	NA
Control			200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.25		
Solvent[1]	0	24	200	0	0	0	0	0	1	0	1	0	1	(0.5)	1	(0.5)	0.13		
MOM	0.005	24	200	0	0	0	0	1	0	0	1	3	1	(0.5)	1	(0.5)	0.13	−	−
MOM	0.010	24	200	0	3	14	0	0	0	0	17	1	10 *	(5.0)	10 *	(5.0)	3.13 *	±	−
MOM	0.020	24	200	1	29	74	1	2	1	0	108	3	41 *	(20.5)	40 *	(20.0)	5.88 *	+	±
MC	0.00005	24	200	3	25	50	3	4	0	0	85	1	59 *	(29.5)	57 *	(28.5)	0.13	+	−
Solvent[1]	0	48	200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.13		
MOM	0.005	48	200	0	0	1	0	0	0	0	1	0	1	(0.5)	1	(0.5)	1.38 *	−	−
MOM	0.010	48	200	0	0	0	0	0	0	0	0	2	0	(0.0)	0	(0.0)	1.00	−	−
MOM	0.020	48	200	1	1	10	0	3	2	10	27	6	12 *	(6.0)	11 *	(5.5)	5.00 *	±	±
MC	0.00005	48	200	4	21	53	2	3	16	0	99	8	59 *	(29.5)	59 *	(29.5)	0.38	+	−

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, MC : mitomycin C.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). * : Significantly different from solvent control at $p < 0.05$. ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

Table 2 Chromosome analysis of Chinese hamster cells (CHL) treated with methoxymethanol. ** with and without S9 mix

Group	Concentration (mg/ml)	S9 mix	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations: gap	ctb	cte	csb	cse	f	mul[2]	total	Others[3]	No. of cells with aberrations: TAG	(%)	TA	(%)	Polyploid[4] (%)	Judgement[5]: SA	NA
Control				200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.50		
Solvent[1]	0	−	6 - (18)	200	0	0	:	0	0	0	0	1	1	1	(0.5)	1	(0.5)	1.50		
MOM	0.005	−	6 - (18)	200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	1.25	−	−
MOM	0.010	−	6 - (18)	200	0	1	2	0	0	0	0	3	0	2	(1.0)	2	(1.0)	3.25 *	−	−
MOM	0.020	−	6 - (18)	200	0	28	67	0	0	.	10	126	0	52 *	(26.0)	52 *	(26.0)	2.65 *	+	−
CPA	0.005	−	6 - (18)	200	2	0	:	0	0	0	0	3	1	3	(1.5)	1	(0.5)	0.13	−	−
Solvent[1]	0	+	6 (18)	200	0	0	:	0	0	0	0	1	1	1	(0.5)	1	(0.5)	0.25		
MOM	0.008	+	6 - (18)	200	1	0	:	0	0	0	0	2	1	2	(1.0)	1	(0.5)	0.13	−	−
MOM	0.016	+	6 - (18)	200	1	0	0	0	0	:	0	2	0	2	(1.0)	1	(0.5)	1.63 *	−	−
MOM	0.032	+	6 - (18)	200	2	16	41	0	1	2	0	62	2	33 *	(16.5)	32 *	(16.0)	5.75 *	+	±
CPA	0.005	+	6 (18)	200	4	32	33	2	0	3	0	64	4	49 *	(24.5)	45 *	(22.5)	0.13	+	−

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, CPA : cyclophosphamide.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). 6) Seven hundred and nineteen-three cells were analysed. * : Significantly different from solvent control at $p < 0.05$ ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

Conclusion :
Under the conditions of this study, it is concluded that methoxymethanol induces chromosomal aberrations to CHL cells in vitro.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (12)

5.6 GENETIC TOXICITY 'IN VIVO'

5.8.1 TOXICITY TO FERTILITY

Type : Fertility
Species : rat
Sex : male/female
Strain : Crj: CD(SD)
Route of admin. : gavage
Exposure period : 14 day premating to lactation day 4
Frequency of treatm. : daily
Premating exposure period
Male : 14 days
Female : 14 days
Duration of test :
No. of generation studies : 2
Doses : 12, 60 or 300 mg/lg-day
Control group : yes, concurrent vehicle
Method : OECD Guide-line 422
Year :
GLP : yes

Test substance : other TS: see freetext

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and

eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS:

Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant

difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

REPRODUCTIVE TOX: All females that copulated resulted in pregnancy and no effect of the administered test substance on either the copulation or fertility indices was recognized. Further, most of the pairs successfully mated during the first estrous stage and there were no significant differences among the pairing days. Also, no histopathological changes were found in the ova of the single female of which copulation was unconfirmed. Reproductive parameters are shown in the table.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : Rerpo.bmp

Table 6 Summary of reproductive performance in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of animals	10	10	10	10
No. of pairs copulated	10	10	10	10
No. of pregnant females	10	10	10	10
Copulation index (%) [a]	100.0	90.0	100.0	100.0
Fertility index (%) [b]	100.0	100.0	100.0	100.0
Pairing days [c]	2.8 ± 1.48	2.4 ± 1.01	3.2 ± 1.62	2.8 ± 1.32
E.S. [d]	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.32	0.0 ± 0.00
(Mean ± S.D.)				

a) (Number of animals with successful copulation/number of animals mated)×100
b) (Number of pregnant animals/number of animals with successful copulation)×100
c) Days between initial pairing and detection of copulation.
d) Number of estrous stages without copulation.

Conclusion :

No adverse effects were seen on reproduction in this screening study.

Reproductive NOAEL 300 mg/kg-day

Parental NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

01.12.2003 (9)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	14 days premating to lactation day 4
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	12, 60 or 300 mg/kg bw-day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 60 mg/kg bw
NOAEL teratogen.	:	= 300 mg/kg bw
NOAEL Fetotoxicity	:	= 60 mg/kg bw
Result	:	Not specific developmental toxin
Method	:	other: OECD Guideline 422
Year	:	
GLP	:	yes
Test substance	:	other TS: see freetext

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300

mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

DEVELOPMENTAL TOX

VIABILITY: A few still births and neonatal deaths occurred in each group, but there was no significant difference between the control group and dose groups regarding the number of pups in the litter, number of live pups, sex ratio, or live birth and viability indices.

EXTERNAL EXAMINATION: No newborn pups showed any external abnormalities in any group and their general condition subsequent to their birth indicated no abnormalities attributable to the administered test substance.

PUP WEIGHTS AND WEIGH GAIN: For both males and females, the weights measured on the lactation days 0 and 4, and the weight increase between these two dates showed no significant difference between the control group and the dose groups.

SKELETAL EXAMINATION: There were no skeletal malformations found in the control or 300 mg/kg-day groups. As variations, excess hypoglossal foramen, closure of the transverse foramen of cervical vertebrae, splitting of the ossification center of vertebral tubercle of the atlas, accessory sternebra, cervical rib, 14th rib (costal vestigium) and a shortening of the 13th rib were noted. These variations were not significantly increased as compared to the control group. Further, the occurrence of accessory sternebra in the 300mg/kg-day group was marginally significant and was considered an incidental finding.

VISCERAL EXAMINATION: There was a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. In the 300 mg/kg-day group, the incidence was 10 pups from 6 litters. Control incidence was 2 pups from 2 litters. One pup form the 60 mg/kg-day group displayed this pathology. Other findings were not dose related and were considered incidental.

VISCERAL EXAMINATION OF DEAD PUPS: The number of early-death pups that were suitable for examination was 1, 3, 2, and 9 pups from the

control, 12 mg/kg-day, 60 mg/kg-day, and the 300 mg/kg-day group, respectively. Among pups found dead on the day of delivery, one high-dose pup had a hydrocephalus. Among those that expired after lactation day 1, one pup each from the control group and the high-dose group showed patent ductus arteriosus, and one pup from the 12 mg/kg group revealed dilatation of the renal pelvis. As there were few findings and no dose-response relationship these effects are considered unrelated to administration of the test substance. Other findings from the animals that died on the day of delivery include, patent foramen ovale was found in one pup from the 12 mg/kg-day group and in 2 pups from the 300 mg/kg-day group. There were also 4 cases of patent ductus arteriosus in the 300 mg/kg-day group. These findings are attributed to the fact that the pups died during parturition resulting in an incomplete closure of either the foramen ovale or ductus arteriosus.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : Develop-Finds.bmp
Develop.bmp

Table 8 Skeletal and visceral findings of pups (F1)from dams (F0)treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg		60 mg/kg		300 mg/kg	
No. of dams	10		10		10	
Skeletal examination						
No. of pups examined	78		$		77	
No. of abnormal pups (%)	15	(18.9)			13	(16.3)
Foramen hypoglossi double	1	(1.3)			0	
Closure of transverse foramen of one or more cervical vertebrae	4	(5.1)			9	(11.1)
Splitting of ossification centers of the ventral tubercle of the atlas	0				1	(1.3)
Accessory sternebrae	5	(6.2)			0	**
Cervical ribs	0				1	(1.4)
14th ribs	2	(2.5)			0	
Reduced 13th ribs	4	(5.0)			2	(2.5)
Visceral examination						
No. of pups examined	72		62		72	
No. of abnormal pups (%)	8	(10.9)	8	(12.2)	16	(22.4)
Thymic remunant in the neck	3	(4.1)	2	(3.3)	3	(4.2)
Deformity of the heart	1	(1.4)	0		0	
Patent foramen ovale	2	(2.7)	1	(1.7)	10	(14.5)*
Patent ductus arteriosus	2	(2.7)	2	(2.9)	3	(3.8)
Supernumerary of the comary orifice	2	(2.7)	0		0	
High take off of the comary orifice	0		0		1	(1.3)
Dilatation of the renal pelvis	0		3	(4.3)**	0	

$: Not examined.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 7 Findings of delivery in dams (F0) treated orally with methoxymethanol and observation on their pups (F1) in combined repeat dose and reproductive/developmental toxicity screening test

Dose level			0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of dams observed			10	9	10	10
No. of dams observed live pups			10	9	10	10
Gestation length			22.7 ± 0.48	22.4 ± 0.73	22.4 ± 0.52	22.2 ± 0.42
No. of corpora lutea			18.1 ± 2.64	17.9 ± 3.92	18.2 ± 4.54	19.3 ± 4.00
No. of implantation sites			16.4 ± 1.35	14.3 ± 4.92	14.6 ± 3.24	16.4 ± 1.84
No. of pups born			15.2 ± 1.55	14.2 ± 5.07	13.6 ± 3.20	15.9 ± 1.73
No. of live pups on day 0			15.2 ± 1.55	14.1 ± 5.04	13.5 ± 3.10	15.2 ± 1.75
Male			7.0 ± 2.40	7.1 ± 3.59	5.7 ± 2.36	6.9 ± 2.33
Female			8.2 ± 2.94	7.0 ± 3.57	7.8 ± 2.53	8.3 ± 2.21
Sex ratio (Male/Female)			0.85 (70/82)	1.02 (64/63)	0.73 (57/78)	0.85 (69/83)
No. of live pups on day 4			15.0 ± 1.49	13.5 ± 5.34	13.1 ± 2.81	14.9 ± 1.66
Male			6.9 ± 2.51	6.9 ± 3.92	5.6 ± 2.22	6.8 ± 2.15
Female			8.1 ± 2.96	6.7 ± 3.16	7.5 ± 2.32	8.1 ± 2.02
Gestation index (%) [a]			100	100	100	100
Implantation index (%) [b]			91.6 ± 9.86	80.4 ± 24.98	81.9 ± 18.70	87.7 ± 16.05
Delivery index (%) [c]			92.7 ± 5.37	91.8 ± 16.20	93.0 ± 7.03	97.1 ± 4.00
Live birth index (%) [d]			100.0 ± 0.00	99.3 ± 2.07	99.4 ± 1.87	95.8 ± 6.35
Viability index (%) [e]			98.7 ± 2.66	86.1 ± 33.33	97.5 ± 4.36	98.1 ± 3.06
Pups body weight						
Male	On day	0	6.9 ± 0.54	6.4 ± 0.55	6.9 ± 0.94	6.2 ± 0.59
		4	11.1 ± 0.99	10.5 ± 0.84	10.9 ± 2.25	10.0 ± 0.85
	Gain	0-4	4.1 ± 0.59	4.1 ± 0.48	4.0 ± 1.45	3.8 ± 0.43
Female	On day	0	6.5 ± 0.60	6.0 ± 0.77	6.6 ± 0.87	5.9 ± 0.47
		4	10.6 ± 0.95	9.9 ± 1.15	10.7 ± 2.04	9.5 ± 0.93
	Gain	0-4	4.1 ± 0.54	3.9 ± 0.50	4.0 ± 1.25	3.6 ± 0.58

a) (Number of females with live pups/number of pregnant females) ×100
b) (Number of total implants/number of total corpora lutea) ×100
c) (Number of total pups/number of total implants) ×100
d) (Number of total live pups on day 0 after birth/number of total pups born) ×100
e) (Number of total live pups on day 4 after birth/number of total live pups on day 0) ×100

Conclusion :

No malformations were observed that were attributable to administration of the test substance. High-dose pups were not different from controls in body weight, sex ratio, mean pup weights, number of pups born, or other similar parameters. Visceral examination revealed a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. This is interpreted as a fetotoxic effect at the high dose associated with a developmental delay.

Developmental NOAEL 60 mg/kg-day

Maternal NOAEL 60 mg/kg-day

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

01.12.2003 (9)

(1) Bills, D. et al.: "Investigation in fish control. 73. Formalin, its toxicity to nontarget aquatic organisms, persistence and counteraction"; Washington DC, U.S. Department of the Interior, Fish and Wildlife Service, 1-7,(1977)

(2) Bringmann,G., Kuehn,R., Vom Wasser 50, 45-60, 1978

(3) Calculation using EPIWIN 3.05 by Toxicology and Regulatory Affairs, October 2003

(4) Calculations and Estimate by Toxicology and Regulatory Affairs, Freeburg IL, using the EPA ECOSAR v0.99f program, November 2003.

(5) Calculations and Estimate by Toxicology and Regulatory Affairs, Freeburg IL, using the EPA ECOSAR v0.99f program, October 2003.

(6) Calculations by Toxicology and Regulatory Affairs, Freeburg IL, using the SRC EPIWIN 3.05 program, October 2003.

(7) Calculations by Toxicology and Regulatory Affairs, Freeburg, IL 62243. Based on reaction kinetics in: Funderburk LH, L Alwdin and W Jencks. Mechanisms of General Acid and Base Catalysis of the Reactions of Water and Alcohols with Formaldehyde. Journal of the American Chemical Society 100:5444-5459 (1978)

(8) Celanese Chemicals Ltd MSDS-049, Published date: 07/24/2002(V2) Formcel , 55% Formaldehyde/35% Methanol, solution.

(9) Combined Repeated Oral Dose and Reproductive/Developmental Toxicity Screening Test of Methoxymethanol Using Rats. Mitsubishi Chemical Safety Institute Ltd., Kashima Laboratory. From Chemicals Evaluation and Research Institute, Japan. Published in Japanese on: http://wwwdb.mhlw.go.jp/ginc/dbfile1/paper/paper4461-52-3d.html

(10) Gerike,P., Gode,P., Chemosphere 21, 799-812, 1990

(11) Harris, J.C. in Lyman W., Reehl, W. and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6

(12) In Vitro Chromosomal Aberration Test of Methoxymethanol on Cultured Chinese Hamster Cells Mitsubishi Chemical Safety Institute Ltd., Kashima Laboratory. From Chemicals Evaluation and Research Institute, Japan. Published in Japanese on: http://wwwdb.mhlw.go.jp/ginc/dbfile1/paper/paper4461-52-3f.html

(13) Reverse Mutation Test Of Methoxymethanol On Bacteria Mitsubishi Chemical Safety Institute Ltd., Kashima Laboratory. From Chemicals Evaluation and Research Institute, Japan. Published in Japanese on: http://wwwdb.mhlw.go.jp/ginc/dbfile1/paper/paper4461-52-3e.html

(14) Single Oral Dose Toxicity Test of Methoxymethanol in Rats. Mitsubishi Chemical Safety Institute Ltd., Kashima Laboratory. From Chemicals Evaluation and Research Institute, Japan. Published in Japanese on: http://wwwdb.mhlw.go.jp/ginc/dbfile1/paper/paper4461-52-3a.html

(15) Swain, H.M. and Somerville, H.J. (1978) Microbial metabolism of methanol in a model activated sludge system, J. Appl. Bacterl. 45:147-151

(16) Tisler, T. and Zagorc-Koncan, J., Water, Air and Soil Pollution 97, 315 - 322, 1997

(17) Vollhardt, Peter (1987) Organic Chemistry WH Freeman publisher NY p 637